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# Properties of Bacteriophage T4 Mutants Defective in Gene 30 (Deoxyribonucleic Acid Ligase) and the rII Gene

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In *Escherichia coli* K-12 strains infected with phage T4 which is defective in gene 30 [deoxyribonucleic acid (DNA) ligase] and in the rII gene (product unknown), near normal levels of DNA and viable phage were produced. Growth of such T4 ligase-rII double mutants was less efficient in *E. coli* B strains which show the "rapid-lysis" phenotype of rII mutations. In pulse-chase experiments coupled with temperature shifts and with inhibition of DNA synthesis, it was observed that DNA synthesized by gene 30-defective phage is more susceptible to breakdown in vivo when the phage is carrying a wild-type rII gene. Breakdown was delayed or inhibited by continued DNA synthesis. Mutations of the rII gene decreased but did not completely abolish the breakdown. T4 ligase-rII double mutants had normal sensitivity to ultraviolet irradiation.

The enzyme deoxyribonucleic acid (DNA) ligase catalyzes the repair of single-strand breaks in DNA duplexes (5, 11, 12, 27, 32). In *Escherichia coli* and in phage T4-infected *E. coli*, the repair of DNA single-strand breaks may constitute an essential step common to the processes of DNA replication (15, 16, 21, 23-26, 29), recombination (1), repair after ultraviolet (UV) or X-ray irradiation (6, 28), and repair after nucleolytic attack (19, 20). Gene 30 is the structural gene for the DNA ligase of phage T4 (9). Under restrictive conditions, the infection of *E. coli* with *amber* (*am*) or *temperature-sensitive* (*ts*) mutants of T4 gene 30 results in limited DNA synthesis and in very low phage yields (14, 21). This indicates that the gene 30 ligase is needed for phage growth although a DNA ligase is also present in uninfected *E. coli* (11, 12, 27). However, the need for the T4 gene 30 ligase can at least be partially overcome if chloramphenicol is added to infected cultures at early times after infection, suggesting that the phage ligase is used in the repair of DNA damage resulting from the action of endonucleases which are synthesized after T4 infection (19, 20).

It was recently observed that *am* and *ts* mutants of T4 gene 30 become viable under the restrictive conditions when the phage rII gene is mutated (4, 8, 17). The effects of rII mutations on the rescue of T4 gene 30 mutants are analogous to those of chloramphenicol (4). It is possible that, in both cases, intracellular endonuclease activity is reduced and the amount of host DNA ligase present is sufficient to support the needs of phage DNA replication and other repair. In this report, we describe the defects of T4 ligase-rII double mutants and their effects on in vivo DNA synthetic processes.

We have observed that *E. coli* K-12 strains support very efficient growth of T4 ligase-rII double mutants. Under restrictive conditions, some defects in phage DNA synthesis can be detected, but these seem to have small or no effects on phage production and sensitivity to UV irradiation. *E. coli* B strains also support the growth of T4 ligase-rII double mutants, but the absence of lysis-inhibition coupled with the defects in DNA synthesis result in shorter growth cycles and lower phage yields. T4 rII mutations decrease, but do not completely abolish, damage to phage DNA resulting from the gene 30 ligase deficiency.

## MATERIALS AND METHODS

**Phage strains.** The T4 mutations used are listed in Table 1. The gene 30 mutants were generously supplied by J. Hosoda, the rII mutants by S. Champe, and the gene 34 and 43 mutants by R. Edgar. The map locations of the rII mutations used in this study were reported by Benzer (2) and Benzer and Champe (3). Double and triple mutants were constructed by genetic recombination and characterized by complementation and recombination tests and by crossing back to wild type and reisolating the single mutants. All phages carrying *am* mutations were grown on *E. coli* CR63; other strains were grown on *E. coli* BB.

**Bacterial strains.** *E. coli* CR63 and K37*str*$^r$ are permissive for T4 *am* mutants (*su*$^+$*1*, *ser*), and *E. coli* BB, S/6, K38*str*$^r$, and K38(λ)*str*$^r$ are restrictive (*su*$^-$) hosts. The *str*$^r$ strains are resistant to 200 μg of streptomycin per ml. *E. coli* K38*str*$^r$ and K37*str*$^r$ were obtained by curing the corresponding λ-lysogenic strains with λi$^{434}$ phage which was generously supplied, with instructions for curing, by M. Gottesman. *E. coli* K38(λ) and K37(λ), the streptomycin-sensitive ancestors of K38*str*$^r$ and K37*str*$^r$, were obtained from N. Zinder via J. Speyer. They are strains S26 and S26Rle, respectively, of Garen et al. (10). *E. coli* CR63 and S/6 were from R. Edgar and BB was from F. Stahl. *E. coli* S/6 is a derivative of *E. coli* B (7).

**Media.** The growth medium used was M9S (3). M9S was supplemented with thymidine-*methyl*-$^3H$ ($^3$H-TdR) in experiments on DNA synthesis. The $^3$H-TdR was purchased from the New England Nuclear Corp., Boston, Mass. Nonradioactive thymidine (TdR) was purchased from Schwarz BioResearch, Inc., Orangeburg, N.Y.

Tris(hydroxymethyl)aminomethane-UV buffer contained 0.001 M $MgCl_2$, 0.085 M NaCl, and 0.001% gelatin in 0.01 M Tris (*p*H 7.5). Other media and methods for growth of bacterial and phage strains and for phage assays were as described by Steinberg and Edgar (31).

**Burst size.** Burst size (phage production per cell) measurements were made in M9S at 30 C as follows. Log-phase cells ($10^8$ in 1 ml) were infected at a multiplicity of 10 with the phage in question. After 8 min of adsorption, T4 antiserum was added (final concentration $k$ = 1/min) to remove unadsorbed phage. At 13 min after infection, the cultures were diluted $10^3$-fold in M9S, and the infective-center titers were determined. The diluted cultures were lysed with $CHCl_3$ and assayed for burst titers after 2 hr of incubation. Phage assays were made on *E. coli* CR63.

**UV irradiation.** The procedure was similar to that used by Speyer and Rosenberg (30). The source of UV light was a filtered Mineralight V-41 lamp (Ultraviolet Products, Inc., San Gabriel, Calif.). Samples (0.2 ml) of phage (at $10^7$/ml) in Tris-UV buffer were placed in duplicate in wells of depression plates (model 96 U-CV, Linbro Chemical Co., Inc., New Haven, Conn.) and irradiated at a dose rate of 5 ergs per mm$^2$ per sec, incident to the suspensions. Four samples were irradiated at a time. The wells containing the samples were placed on a turning Spray-Fisher dish turntable (Fisher Scientific Co., Springfield, N.J.) during irradiation to allow identical exposure of all phage suspensions. The irradiation was carried out in subdued light, but all other manipulations preceding the platings for survivors were carried out under normal laboratory fluorescent lighting. Platings were done under conditions of yellow light from a G.E. Gold fluorescent lamp which does not cause photoreactivation (13). The concentration of agar in the top layer for plating was 0.5% instead of the usual 0.65% (31). This "softer" agar permits faster diffusion of phage on the plates and allows for better visibility of plaques when phage development is delayed because of UV damage. The plates were incubated at 30 C in total darkness.

## RESULTS

**Plating properties of T4 ligase-rII double mutants.** Table 2 shows that the T4 ligase-rII double mutants *am*H39X/rUV375 and *am*E605/rUV375 plated at high efficiency on *E. coli* strains which restrict *am*H39X, *am*E605, and other T4 *am* mutations. These double mutants also failed to grow on λ lysogens, indicating that ligase mutations do not, in turn, suppress the rII mutant phenotype. The plaques formed by ligase-rII

TABLE 1. *Phage T4 mutants*

| Gene | Mutant designation |
|---|---|
| 30 | *am*H39X, *am*E605 |
| 34 | *am*B265 |
| 43 | *am*B22, *ts*L56 |
| rII | *r*UV375(rIIB ochre), *r*205(rIIA missense or frameshift), *r*638(rIIB deletion), *r*1605(rIIA–rIIB deletion) |

TABLE 2. *Effect of T4 rII mutations on the plating efficiency of gene 30 mutants*

| Mutant | Plating efficiency (%)[a] on *E. coli* | | | | |
|---|---|---|---|---|---|
| | K37*str*$^r$ | K38*str*$^r$ | BB | S/6 | K38(λ)*str*$^r$ |
| *am*H39X | 100 | $5 \times 10^{-2}$ | $4 \times 10^{-2}$ | $2 \times 10^{-4}$ | $10^{-3}$ |
| *am*H39X/*r*UV375 | 100 | 94 | 90 | 0.1 | $<10^{-6}$ |
| *am*E605 | 100 | $5 \times 10^{-2}$ | $5 \times 10^{-2}$ | $10^{-4}$ | $5 \times 10^{-4}$ |
| *am*E605/*r*UV375 | 100 | 84 | 104 | $3 \times 10^{-2}$ | $<10^{-6}$ |
| *am*B22 | 100 | $3 \times 10^{-6}$ | $9 \times 10^{-6}$ | $3 \times 10^{-6}$ | $2 \times 10^{-6}$ |
| *am*B22/*r*UV375 | 100 | $7 \times 10^{-6}$ | $4 \times 10^{-6}$ | $4 \times 10^{-6}$ | $<10^{-6}$ |
| *am*B265 | 100 | $4 \times 10^{-5}$ | $6 \times 10^{-5}$ | $6 \times 10^{-5}$ | $4 \times 10^{-5}$ |
| *am*B265/*r*UV375 | 100 | $5 \times 10^{-5}$ | $8 \times 10^{-5}$ | $6 \times 10^{-5}$ | $<10^{-6}$ |
| *r*UV375 | 100 | 108 | 92 | 61 | $8 \times 10^{-6}$ |

[a] Plating efficiencies were determined relative to K37*str*$^r$.

double mutants on *E. coli* BB and K38*str*$^r$ are slightly smaller than wild-type plaques, and on *E. coli* K37 *str*$^r$ they are indistinguishable from wild type. On *E. coli* S/6, however, all of the ligase-rII double mutants which we have tested produced very small plaques which were often barely visible. The apparent low plating efficiency of *am*H39X/rUV375 and *am*E605/rUV375 on *E. coli* S/6 (Table 2) is probably due to inaccuracies in plaque counts rather than to the inability of these double mutants to adsorb to S/6 or to propagate in these cells; the transmission coefficient of *am*H39X/rUV375-infected *E. coli* S/6 is near unity and is about the same as that of S/6 infected with rUV375.

The results in Table 3 show the effectiveness of rII mutations in overcoming the need for the gene 30 ligase. Ligase-rII double mutants gave phage yields near control values when grown in the *am*-restricting host, *E. coli* K38*str*$^r$. On the other hand, the burst sizes on *E. coli* S/6 were lower and probably account, in part, for the tiny plaques produced by these double mutants on this bacterial strain. In this experiment (Table 3) and in several similar experiments, the appearance of viable intracellular phage began at about 15 min after infection in all cultures, irrespective of the bacterial host or phage mutant used. However, viable phage yields were consistently lower in *am*H39X/rUV375-infected *E. coli* S/6 cultures, although these experiments were carried out under conditions which do not permit lysis-inhibition of infected *E. coli* K38*str*$^r$. Thus, it appears that ligase-rII phage maturation is more defective in *E. coli* S/6.

**DNA synthesis by ligase-rII double mutants in E. coli S/6.** Figure 1 compares the DNA synthesis profiles for *E. coli* K38*str*$^r$ and S/6 after infection with rUV375, *am*H39X/rUV375, and *am*H39X. The differences between rII, ligase-rII, and ligase

TABLE 3. *Phage production by E. coli infected with T4 ligase-rII double mutants*

| Mutant | Phage production in *E. coli* | | | |
|---|---|---|---|---|
| | K37*str*$^r$ | K38*str*$^r$ | S/6 | K38(λ)*str*$^r$ |
| *am*H39X | 200 | 0.25 | 0.1 | 0.1 |
| *am*H39X/*r*UV375 | 109 | 80 | 10 | 0.06 |
| *am*H39X/*r*1605 | 154 | 177 | 20 | 0.04 |
| *am*H39X/*r*205 | 218 | 146 | 23 | 0.1 |
| *am*H39X/*r*638 | 118 | 114 | 33 | 0.07 |
| *am*B265 | 290 | 0.06 | 0.02 | 0.2 |
| *am*B265/*r*UV375 | 172 | 0.01 | 0.01 | 0.03 |
| *r*UV375 | 136 | 92 | 120 | 0.02 |

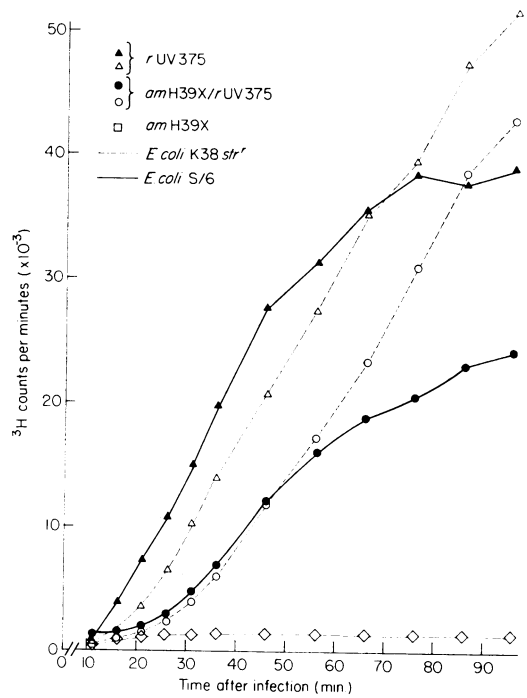


FIG. 1. *DNA synthesis after infection of E. coli K38str*$^r$ *and S/6 with ligase-defective T4. Log-phase cells (10*$^8$ *in 1 ml) were infected at 30 C with 10*$^9$ *particles (in 0.1 ml) of the phage in question. At 6 min after infection, the cultures were diluted 10-fold into M9S containing* $^3$*H-TdR (10 μCi of* $^3$*H per ml at a specific activity of 20 μCi of* $^3$*H per μg of TdR). The incorporation of* $^3$*H-TdR into DNA was measured by the method previously described (17, 18). Infective-center titers were determined on samples treated with anti-T4 serum (k = 1/min) at 6 min after infection. Samples were lysed with* $CHCl_3$ *at 180 min after infection and titered for progeny phage yields.*

mutants are similar to what was previously observed with *E. coli* BB*str*$^r$ (23). DNA synthesis in in the cells infected with *am*H39X/rUV375 occurred in two stages: an early stage of *slow* synthesis followed by a second stage of *rapid* synthesis. The length of the early *slow* DNA synthetic period obtained with *am*H39X/rUV375-infected *E. coli* S/6 is very similar to that obtained with the *E. coli* K38*str*$^r$ host. Differences between the two infected bacterial hosts begin to show at later times after infection and are primarily due to earlier lysis of the *E. coli* S/6 cultures.

The experiment shown in Fig. 1 was intentionally carried out under conditions which would permit lysis-inhibition. We found that, in M9S at 30 C and at cell concentrations of about $10^8$ per ml, the addition of 10 rII phage particles per cell resulted in the infection of more than 99% of the cells in 6 min. However, a substantial fraction of

the added phage (40 to 50%) was still not adsorbed by this time. The 10-fold dilution into $^3$H-TdR medium slowed down the rate of adsorption which then continued for at least 50 min, causing lysis-inhibition of the infected *E. coli* K38*str*$^r$ cultures. We observed that cell lysis in the *am*H39X/rUV375-infected S/6 culture began at about 40 min after infection and was nearly complete by 80 min. In the corresponding K38*str*$^r$ culture, the release of phage from infected cells began at about 90 min after infection and lysis was not complete until about 180 min after infection. The burst sizes in this experiment were measured at 180 min after infection were as follows: for *E. coli* host K38*str*$^r$, rUV375, 144; *am*H39X/rUV375, 120; *am*H39X, 0.5; for *E. coli* host S/6, rUV375, 100; *am*H39X/rUV375, 25.

Thus, despite the early slow DNA synthetic activity, *am*H39X/rUV375-infected K38*str*$^r$ cells produced enough DNA to result in a high phage yield. A combination of early slow DNA synthesis and early cell lysis seemed to be the cause for the lower phage yields in the S/6 culture. Weak growth of ligase-rII double mutants was also observed with all of several derivatives of *E. coli* B which show the rapid-lysis phenotype of rII mutations. The two-step DNA synthesis profile (Fig. 1) has been observed with all the ligase-rII double mutants which we have tested, including cases where rII deletions were used. Thus, the early slow DNA synthetic activity is independent of the quality of the mutated rII gene product as is probably caused by the gene 30 ligase deficiency.

**Stability of replicating DNA in cells infected with T4 ligase-rII double mutants.** The T4 gene 43 (DNA polymerase) mutation *ts*L56 was introduced into *am*H39X/rUV375, *am*H39X, and rUV375 phages. The presence of this mutation did not alter qualitatively the shapes of the profiles shown in Fig. 1 when growth was at 30 C. A shift to 42 C stopped DNA synthesis rapidly. It has been observed that, after DNA synthesis starts at 30 C in *ts*L56-infected cells, a shift to 42 C and maintenance at this temperature result in gradual loss of trichloroacetic acid-insoluble radioactivity from phage DNA (18). We assume here that this solubilization of counts reflects the suspectibility of phage DNA to nucleolytic attack in vivo and suggests the presence of single-strand breaks and gaps in replicated phage DNA.

T4 mutants carrying various combinations of *ts*L56, *am*H39X, and rUV375 were used to infect *E. coli* K38*str*$^r$ and were permitted to begin synthesizing DNA at 30 C. An 8-min pulse of $^3$H-TdR was given to the infected cultures at the permissive temperature to label DNA within the period corresponding to the *slow* rate of DNA synthesis in cells infected with ligase-rII mutants. After the pulse, the cultures were diluted into excess nonradioactive TdR medium at 42 C, and the fate of the counts previously incorporated at 30 C was followed (Fig. 2). The following observations can be made with respect to the effect of the rII mutation: (i) DNA made by *am*H39X/rUV375 was more stable than DNA made by *am*H39X-(rII+), and (ii) DNA made by *am*H39X/rUV375/*ts*L56 was more stable than DNA made by *am*H39X/*ts*L56(rII+). Thus, the rII mutation resulted in increased stability of replicated phage DNA. Figure 2 also shows that, after the shift-up

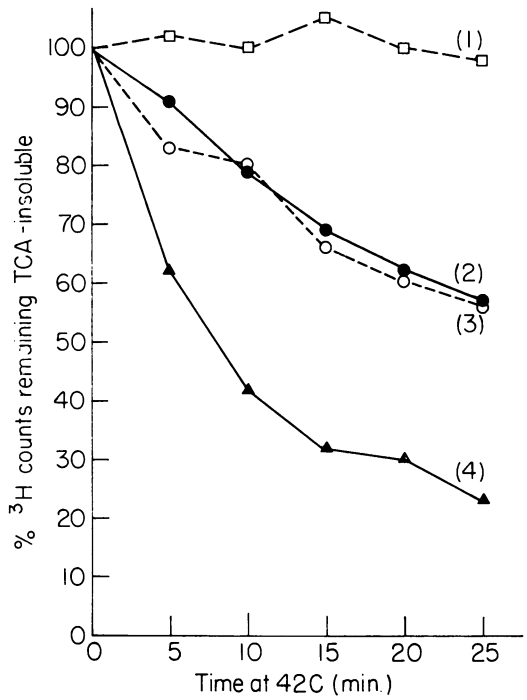


FIG. 2. *Stability of DNA replicated by ligase-defective T4. Log-phase E. coli K38str*$^r$ *cells (10*$^8$ *in 1 ml) were infected at 30 C with 10*$^9$ *particles (in 0.1 ml) of the phage in question and incubated with occasional mixing. At 12 min after infection, 0.2 ml of each infected culture was transferred to 20 μliters of* $^3$*H-TdR medium containing 2 μCi of* $^3$*H at a specific activity of 27.8 μCi of* $^3$*H per μg of TdR. After 8 min of incubation at 30 C with* $^3$*H-TdR, 0.15 ml of each incorporating culture was transferred to 42 C by diluting in 0.6 ml of prewarmed M9S containing 200 μg of TdR per ml. The amount of trichloroacetic acid-insoluble* $^3$*H was determined on samples taken within 30 sec after dilution at 42 C (zero time on the graph) and at subsequent 5-min intervals. The amounts of trichloroacetic acid-insoluble* $^3$*H present at zero time were as follows: (1) amH39X/rUV375, 2,617 counts/min; (2) am H39X/rUV375/tsL56, 1,252 counts/min; (3) amH39X, 1,574 counts/min; (4) amH39X/tsL56, 1,236 counts/min.*

to 42 C, DNA made by *am*H39X/*r*UV375/*ts*L56 was unstable whereas DNA made by *am*H39X/*r*UV375 was quite stable. In the experiments with *am*H39X/*r*UV375 and *am*H39X, DNA synthesis was presumably not halted by the temperature shift since both of these phages carry the wild-type allele for gene 43. However, $^3$H-TdR incorporation stopped because of the chase with nonradioactive substrate. In cells infected with phage carrying the *ts*L56 mutation, DNA synthesis and the potential to incorporate $^3$H-TdR were stopped by the chase at 42 C. Other *ts* mutations in gene 43 and in genes 32 and 45 lead to effects similar to those of *ts*L56 (*unpublished data*). So the difference in DNA stabilities between *am*H39X/*r*UV375/*ts*L56 and *am*H39X/*r*UV375 indicates that the continuation of DNA synthesis is necessary to maintain the stability of phage DNA in cells infected with ligase-rII double-mutants.

Figure 3 compares the stabilities of DNA made by *am*H39X/*r*UV375/*ts*L56 and *r*UV375/*ts*L56 (normal gene 30 ligase) during the *rapid* phase of DNA synthesis. The experiment is very similar to the one described in Fig. 2; a shorter pulse period (2 min) was used here to compensate, in part, for the difference in the rates of DNA synthesis during early and late times after infection. Figure 3 shows that DNA made at later times after infection in the absence of the gene 30 ligase (*am*H39X/*r*UV375/*ts*L56) was more susceptible to breakdown than DNA made by the phage carrying a normal gene 30 (*r*UV375/*ts*L56). This means that phage DNA replication is affected by the gene 30 ligase deficiency throughout the phage growth cycle and that the transition from *slow* to *rapid* synthesis is probably not a reflection of increased DNA repair activity during late times after infection.

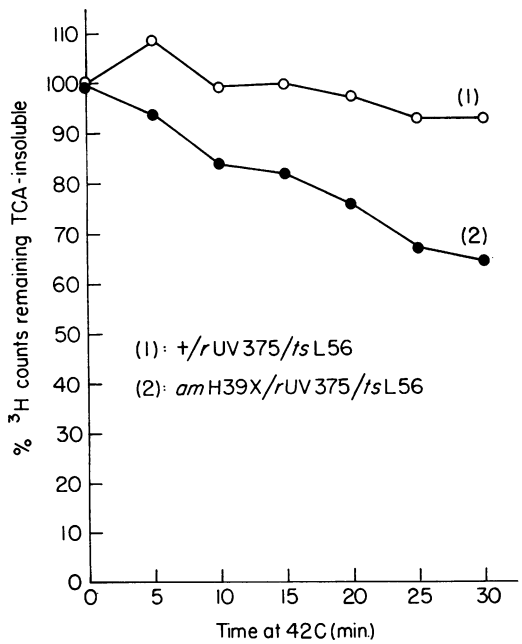


FIG. 3. *Stability of DNA replicated by amH39X/rUV375/tsL56 late after infection. The conditions were as described in Fig. 2, except that the pulse with $^3$H-TdR was for 2 min beginning at 38 min after infection. The amounts of trichloroacetic acid-insoluble $^3$H present at zero time were 3,738 counts/min for amH39X/rUV375/tsL56 and 5,096 counts/min for rUV375/tsL56.*

**UV sensitivity of T4 ligase-rII double mutants.** Figure 4 compares the UV sensitivities of *r*UV375 and *am*H39X/*r*UV375. The two phages have very similar survival curves, implying that the gene 30 ligase is not essential for the in vivo repair of UV-irradiated DNA when the rII gene is mutated. In other experiments (not shown), we have observed that rII mutants are no more sensitive to UV irradiation than wild-type phage.

## DISCUSSION

In *E. coli* infected with phage T4 rII mutants, the phage DNA ligase (gene 30) is not essential for DNA replication and phage production (4, 14). T4 ligase-rII double mutants are restricted in *E. coli* K-12 (λ) and grow poorly in *E. coli* S/6 (a derivative of *E. coli* B). The poor growth of

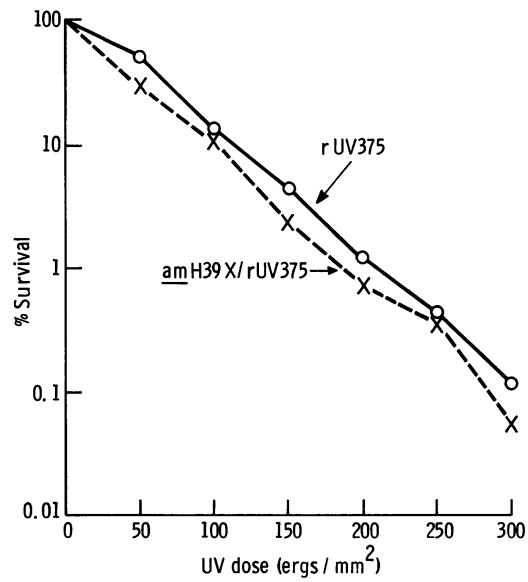


FIG. 4. *UV sensitivity of T4 ligase-rII double mutants. Phage survival after UV irradiation was determined by the method described in Materials and Methods.*

these double mutants on *E. coli* B strains is due to a combination of defective DNA synthesis and to cell lysis which occurs before the accumulation of large amounts of intracellular phage particles. *E. coli* strains which do not show the rapid-lysis phenotype of rII mutations (e.g., K38*str*$^r$ and BB) produce near normal yields of phage after infection with T4 ligase-rII double mutants despite abnormalities in DNA synthesis.

In pulse-chase experiments, we have shown that DNA synthesized by gene 30-defective phage is more susceptible to breakdown in vivo if the phage is carrying a wild-type rII gene. Our experiments (Fig. 2) also show that the continuation of DNA replication after infection stabilizes DNA against degradation despite the gene 30 defect in the double mutants. This suggests that DNA replication involves the repair of single-strand gaps as well as breaks. The repair may be carried out by host-derived or phage-induced enzymes, including an alternate ligase activity. If such a substitution does occur, then it must be very efficient since the DNA synthetic capacity of cells infected with ligase-rII double mutants is very similar to that of cells infected with phage carrying a normal ligase gene (Fig. 1). Also, the survival of UV-irradiated phage, which presumably requires ligase activity (28), is not altered when gene 30 is defective (Fig. 4).

It has been observed that, in *su*$^-$ cells infected with T4 *am* gene 30 mutants, the addition of chloramphenicol at early times after infection leads to the inhibition of phage DNA breakdown and to productive DNA replication (19, 20). The effects of chloramphenicol, however, may be somewhat different from those of rII mutations (16), since this antibiotic inhibits all protein synthesis (DNA synthetic as well as degradative enzymes) whereas rII mutations probably affect only some functions. The effect of rII mutations in the rescue of ligase-defective T4 may be twofold: (i) to decrease endonuclease activity, directly or indirectly, and (ii) to make host repair activities (possibly the host ligase) more accessible for T4 DNA replication and repair.

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